

We claim:

1           1.     A method for measuring the activity of intracellular chemical  
2 reactions in a cell comprising:

3           providing substrate molecules containing a label, the labeled substrate  
4           molecules specific to a chemical reaction whose activity is to be  
5           measured;

6           disposing said substrate molecules within a single cell;

7           allowing said substrate molecules within the single cell to take part in the  
8           chemical reaction to produce altered substrate molecules;

9           liberating said substrate molecules and said altered substrate molecules  
10          from the single cell;

11          detecting the label to identify the substrate molecules and the altered  
12          substrate molecules from the single cell; and

13          determining activity of said chemical reaction from a comparison of  
14          detected altered substrate molecules with detected substrate  
15          molecules.

1           2.     The method of claim 1 further comprising quantifying the amounts  
2 of detected altered substrate molecules and detected substrate molecules.

1           3.     The method of claim 1 wherein said intracellular chemical reaction  
2 in said cell comprises enzyme catalysis.

1                   4.     The method of claim 1 wherein said altered substrate molecules  
2 exhibit a change in electrophoretic mobility as compared with the substrate molecules.

1                   5.     The method of claim 4, wherein detecting said substrate molecules  
2 and said altered substrate molecules comprises electrophoresis.

1                   6.     The method of claim 2 wherein quantifying the amounts of detected  
2 altered substrate molecules and detected substrate molecules comprises detection of  
3 the label by fluorescence following separation by electrophoresis.

1                   7.     The method of claim 1 wherein disposing said substrate molecules  
2 within a single cell comprises using a naturally occurring substrate molecule within said  
3 cell, inducing said substrate molecule to be produced within said cell, or introducing  
4 said substrate molecule into said cell from outside said cell.

1                   8.     The method of claim 7 wherein introducing said substrate  
2 molecules into said cell from outside said cell comprises microinjecting, electroporating,  
3 optoporating, vesicle fusing, pinocytic loading, or associating said substrate molecules  
4 with membrane permeant peptides.

1                   9.     The method of claim 1 further comprising stimulating said cell while  
2 said substrate molecules are intracellularly present prior to liberating said substrate  
3 molecules and said altered substrate molecules from the single cell.

10. The method of claim 9 further comprising comparing activity of said  
chemical reaction with a similar activity determined from a single cell that has not been  
stimulated.

11. The method of claim 1, wherein liberating said substrate molecules  
and said altered substrate molecules from the single cell comprises chemical disruption  
of said single cell, mechanical disruption of said single cell, or separation of said  
substrate and altered substrate molecules by electrophoresis, or by a combination  
thereof.

12. The method of claim 1, wherein the label is selected from a group  
consisting of fluorescent labels, isotopes, labels exhibiting optical absorption, and  
electron spin resonance labels.

13. The method of claim 1 wherein the substrate molecules are  
polymers.

14. The method of claim 13 wherein the polymers are selected from a  
group consisting of peptides, polysaccharide, and nucleic acids.

15. The method of claim 14 wherein said polymers are modified with a  
fluorescent label.

1           16. The method of claim 14 wherein said peptides are substrates for a  
2 kinase that alters said modified peptides by the addition of a phosphate moiety to a  
3 particular amino acid within each peptide.

1           17. The method of claim 16, wherein said peptide has been modified  
2 by covalent addition of a fluorescent group.

1           18. The method of claim 1, said substrate molecules comprise  
2 carbohydrates, phospholipids, entire proteins, or organic compounds not ordinarily  
3 found within the cell.

1           19. The method of claim 1 wherein detecting the label comprises  
2 performing voltammetry or mass spectrometry.

1           20. The method of claim 1 further comprising simultaneously  
2 performing each of said steps with a plurality of different substrate molecules, each  
3 reporting on a specific chemical reaction within a single cell.

1           21. A method for measuring activity of a chemical reaction in a minute  
2 volume of tens of pl or less comprising:

3           providing substrate molecules containing a label;

4 disposing said substrate molecule into said minute volume where said  
5 chemical reactions occurs producing altered substrate molecules  
6 within said minute volume;  
7 terminating said chemical reactions;  
8 detecting the label to identify the substrate molecules and the altered  
9 substrate molecules to determine activity of the chemical reaction.

1 22. The method of claim 21 further comprising quantifying changes in  
2 the amounts of the substrate molecules and the altered substrate molecules.

1 23. An apparatus for measuring an activity of chemical reactions of  
2 intracellular molecules comprising:

3 means for disposing labeled substrate molecules into said cell to form  
4 labeled altered substrate molecules therein; and  
5 means for detecting said substrate molecules and said altered substrate  
6 molecules from a single cell before any substantial alteration of  
7 said substrate molecules and said altered substrate molecules has  
8 occurred.

1 24. The apparatus of claim 23 further comprising means for quantifying  
2 changes in the amounts of said substrate molecules and said altered substrate  
3 molecules.

1           25. The apparatus of claim 23 wherein said means for detecting  
2 detects enzyme catalysis.

1           26. The apparatus of claim 23 wherein said means for detecting  
2 detects a change in electrophoretic mobility of said substrate molecules versus said  
3 altered substrate molecules.

1           27. The apparatus of claim 23 wherein said means for detecting  
2 comprises capillary electrophoresis.

1           28. The apparatus of claim 24 wherein said means for quantifying  
2 changes in the amounts of said substrate molecules and said altered substrate  
3 molecules comprises means for quantifying fluorescence of said substrate molecules  
4 and said altered substrate molecules following separation of said substrate molecules  
5 and said altered substrate molecules by capillary electrophoresis.

1           29. The apparatus of claim 23 wherein said means for disposing  
2 comprises means for inducing said substrate molecules to be produced within said cell,  
3 or means for introducing said substrate molecule into said cell from outside said cell.

1           30. The apparatus of claim 29 wherein said means for introducing said  
2 substrate molecules into said cell from outside said cell comprises means for  
3 microinjecting, means for electroporating, means for optoporating, means for vesicle  
4 fusing, means for pinocytic loading, or means for associating said substrate molecules  
5 with membrane permeant peptides.

1           31. The apparatus of claim 23 wherein said means for disposing said  
2 substrate molecules comprises means for providing said substrate molecules from  
3 naturally occurring compounds or synthetically derived compounds.

1           32. The apparatus of claim 23 further comprising means for stimulating  
2 said cell while said substrate molecules are present intracellularly prior to detecting said  
3 substrate molecules and said altered substrate molecules.

1           33. The apparatus of claim 32 wherein said means for detecting  
2 comprises means for obtaining the contents of said cell, and means for separating part  
3 or all of said contents by capillary electrophoresis

1           34. The apparatus of claim 33, wherein said means for obtaining the  
2 contents comprises chemical means for disruption, physical means for disruption,  
3 separation of molecules by electrophoresis, or a combination thereof.

1           35. The apparatus of claim 23 wherein said means for disposing  
2 provides a substrate molecules that are specific for a particular intracellular chemical  
3 reaction.

1           36. The apparatus of claim 35 wherein said means for disposing  
2 provides substrate molecules which are fluorescent.

1           37. The apparatus of claim 36 wherein said means for disposing  
2 provides substrate molecules which are modified peptides.

1           38. The apparatus of claim 37, said modified peptides are substrates  
2 for a kinase that alters said modified peptides by the addition of a phosphate moiety to  
3 a particular amino acid within each said peptide.

1           39. The apparatus of claim 37 wherein said peptides have been  
2 modified by covalent addition of a fluorescent group to allow detection by fluorescence.

1           40. The apparatus of claim 23 wherein said means for disposing  
2 provides substrate molecules comprising nucleic acids, carbohydrates, phospholipids,  
3 entire proteins, or compounds not ordinarily found within cells.



1           41. The apparatus of claim 23 wherein said means for detecting  
2 comprises means for performing voltammetry or means for mass spectrometry on said  
3 substrate molecules and said altered substrate molecules.

1           42. The apparatus of claim 23 further comprising means for  
2 simultaneously disposing and detecting a plurality of different substrate molecules,  
3 each different substrate molecule reporting on a specific chemical reaction.

1           43. An apparatus for measuring an activity of chemical reactions of  
2 molecules in a minute volume of the order of tens of pl or less comprising:

3           means for disposing substrate molecules having a label into said minute  
4 volume for chemical reactions to occur producing altered substrate  
5 molecules; and

6           means for detecting the label to identify the substrate molecules and the  
7 altered substrate molecules to determine activity of the chemical  
8 reaction.

1           44. The apparatus of claim 43 further comprising means for quantifying  
2 changes in the amounts of said substrate molecules and said altered substrate  
3 molecules.

1           45. An apparatus for measuring an activity of intracellular chemical  
2 reactions of molecules in a cell in which labeled substrate molecules have been  
3 disposed to allow for an in vivo reaction wherein labeled altered substrate molecules  
4 are formed, comprising:

5           a detector of said labeled substrate molecules and said labeled altered  
6           substrate molecules; and

7           a cell lysing device communicating with said detector, which cell lysing  
8           device extracts said substrate and altered substrate molecules  
9           from said cell, and which cell lysing device collects and transfers  
10           said substrate and altered substrate molecules into said detector  
11           before any substantial alteration occurs.

1           46. The apparatus of claim 45 further comprising a data processor  
2 coupled to said detector to quantify changes in the amounts of said substrate molecules  
3 and said altered substrate molecules.

1           47. An apparatus for measuring an activity of intracellular chemical  
2 reactions of molecules in a cell in which labeled substrate molecules have been  
3 disposed to allow for an in vivo reaction wherein labeled altered substrate molecules  
4 are formed, comprising:

5           means for holding the cell;

an electrophoresis reservoir contiguous to but not in fluidic contact with  
said means for holding;  
a sharpened electrophoresis capillary for puncturing the cell to remove a  
cellular sample;  
means for moving said electrophoresis capillary to puncture said cell;  
means for rapidly transitioning said capillary into contact with said  
electrophoresis reservoir after removing said cellular sample so  
that electrophoresis of said cellular sample through said capillary  
will commence; and  
a detector for detecting said labeled substrate molecules and said labeled  
altered substrate molecules during or following electrophoresis.